Remarks

Claims 3-4, 7, 13, 15, and 17 have been canceled. Claims 1, 6, 14, and 18 have been amended. No new matter is added, and support for the amendments is found throughout the specification and in the original claims. By entry of this amendment, Claims 1-2, 5-6, 8-12, 14, 16, and 18-24 are pending.

Applicants wish to thank the Examiner for conducting a telephonic interview with applicants' counsel, Jamie Greene, on July 29, 2005. Differences between the claimed method and the method of *Seeger et al.* were discussed during the interview. Differences between the techniques of surface plasmon resonance and FRET were also discussed, and documents summarizing these two methods are enclosed with this response. Claims 1 and 6 have been amended as suggested by the Examiner to combine steps (a) and (b) into a single step.

Rejections under 35 U.S.C. §102

In the Office Action mailed March 23, 2005, the Examiner rejected Claims 1-2, 5-6, 9, 12, 22-24 under 35 U.S.C. §102(b) as anticipated by Seeger *et al.* (*Biotechniques*, 1997, 23(3):512-514, 516 and 517, "Seeger"). Applicants respectfully traverse the rejection.

Seeger discloses a purification process that uses peptide nucleic acid (PNA) probes to form triplexes with genomic DNA from blood. The isolated triplex DNA is then purified with magnetic beads, and the DNA is subsequently amplified by PCR using specific primers to genes of interest. Seeger fails to introduce a purine-rich region into the target DNA sequence, and fails to detect the presence of triplex structures formed by the product of the amplification reaction and the peptide nucleic acid.

In the method taught by Seeger, purine rich regions must already be present in the genomic DNA to be captured by PNA. The capture by PNA takes place before the PCR step. Claim 1 is directed to a target sequence in which a purine rich region is added during the amplification step. The target sequence in the claimed methods would be unable to bind PNA

prior to the PCR step. In Claim 6, the target sequence contains a purine region, but the target is introduced to PNA during or after amplification, not before.

Figures 1 on page 514 demonstrates that Seeger is not attempting to detect PNA_2:DNA triplexes as claimed in the instant invention. Figure 1, lanes 8 and 11, of Seeger show sequences amplified in the absence of PNA and lanes 9, 10, 12, and 13 show sequences amplified in the presence of PNA. If triplexes were being detected, then lanes 9, 10, 12, and 13 should have migrated at a slower rate on the gel than lanes 8 and 11 due to a triplex forming between DNA and PNA. There is no disclosure in the Seeger paper, nor is it inherent from the data, that one could uses the method taught by Seeger to detect triplex structures.

Claims 1 has been amended to clarify that the detected triplex structures result from the binding of the amplified target sequence to the peptide nucleic acid, and Claims 6 and 18 have been amended to clarify that the triplex structures to be detected are formed by the product of the amplification reaction and the peptide nucleic acid.

For at least the above reasons, applicants respectfully submit they have overcome the Examiner's rejection under 35 U.S.C. §102(b) and request withdrawal thereof.

Rejections under 35 U.S.C. §103

Claims 14-16 and 18-21 have been rejected under 35 U.S.C. §103(a) as obvious over Seeger in view of Felgner *et al.* (United States Patent No. 6,165,720, "Felgner"). Applicants respectfully traverse.

Claims 14-16 and 18-21 are directed to a kit and method for detecting the presence of a target nucleic acid in a sample. Seeger has been discussed above. The claimed kit and method use an evanescent wave detector apparatus, on which is immobilize a peptide nucleic acid, to detect the presence of a target nucleic acid.

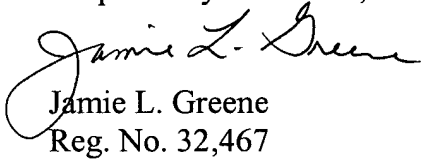
Felgner discloses the use of a spectrofluorometer with FRET in solution. Felgner fails to discuss use of a surface plasmon resonance detector. FRET and surface plasmon resonance detectors are not the same devices. A spectrofluorometer is used to detect fluorescent light emissions, which may be simply from a solution (as described in Example 4). In contrast, a surface plasmon resonance detector detects evanescent waves, which are deflected at a surface differently depending upon the nature of material immobilized on the surface. As mentioned above, documents summarizing FRET and surface plasmon resonance detectors are enclosed with this response as Exhibits A and B, respectively.

Applicants respectfully submit that, for a rejection under 35 U.S.C. §103 to be properly founded, each and every element of the claims must be disclosed in the combination of cited art references. Neither Seeger or Felgner discloses the use of an evanescent wave detector apparatus to detect the presence of a target nucleic acid in a sample. One of ordinary skill in the art lack the motivation motivated to make or use the claimed kit or method in view of the disclosure by Seeger and Felgner. Therefore, withdrawal of this rejection is respectfully requested.

Conclusion

Applicants submit that the pending claims define novel and patentable subject matter and provide a complete response to the Office Action. Accordingly, Applicants respectfully request allowance of these claims. No additional fees are believed due, however, the Commissioner is hereby authorized to charge any deficiencies which may be required, or credit any overpayment, to Deposit Account Number 11-0855.

Respectfully submitted,



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Fluorescence resonance energy transfer

From Wikipedia, the free encyclopedia.
(Redirected from Fluorescence Resonance Energy Transfer)

Fluorescence resonance energy transfer (or **Förster resonance energy transfer**) describes an energy transfer mechanism between two fluorescent molecules. A fluorescent donor is excited at its specific fluorescence excitation wavelength. By a long-range dipole-dipole coupling mechanism, this excited state is then nonradiatively transferred to a second molecule, the acceptor. The donor returns to the electronic ground state. The described energy transfer mechanism is termed "Förster resonance energy transfer" (FRET), named after the German scientist Theodor Förster. When both molecules are fluorescent, the term "fluorescence resonance energy transfer" is often used, although the energy is not actually transferred by fluorescence.

Theoretical basis

The FRET efficiency is determined by three parameters:

1. The distance between the donor and the acceptor.
2. The spectral overlap of the donor emission spectrum and the acceptor absorption spectrum.
3. The relative orientation of the donor emission dipole moment and the acceptor absorption dipole moment.

The FRET efficiency E, which is defined as

$$E = 1 - \tau'_D / \tau_D$$

where τ'_D and τ_D are the donor fluorescence lifetimes in the presence and absence of an acceptor, respectively, or as

$$E = 1 - F_D / F_D$$

where F_D and F_D are the donor fluorescence intensities with and without an acceptor, respectively. E depends on the donor-to-acceptor separation distance r with an inverse 6th order law due to the dipole-dipole coupling mechanism:

$$E = 1 / (1 + (r / R_0)^6)$$

with R_0 being the Förster distance of this pair of donor and acceptor at which the FRET efficiency is 50%. The Förster distance depends on the overlap integral of the donor emission spectrum with the acceptor absorption spectrum and their mutual molecular orientation as expressed by the following equation:

$$R_0^{\,6} = 8.8 \times 10^{23}\ \kappa^2 n^{-4} Q_0 J$$

where κ^2 is the dipole orientation factor, n is the refractive index of the medium, Q_0 is the fluorescence quantum yield of the donor in the absence of the acceptor, and J is the spectral overlap integral calculated as

$$J = \int f_D(\lambda)\, \epsilon_A(\lambda)\, \lambda^4\, d\lambda$$

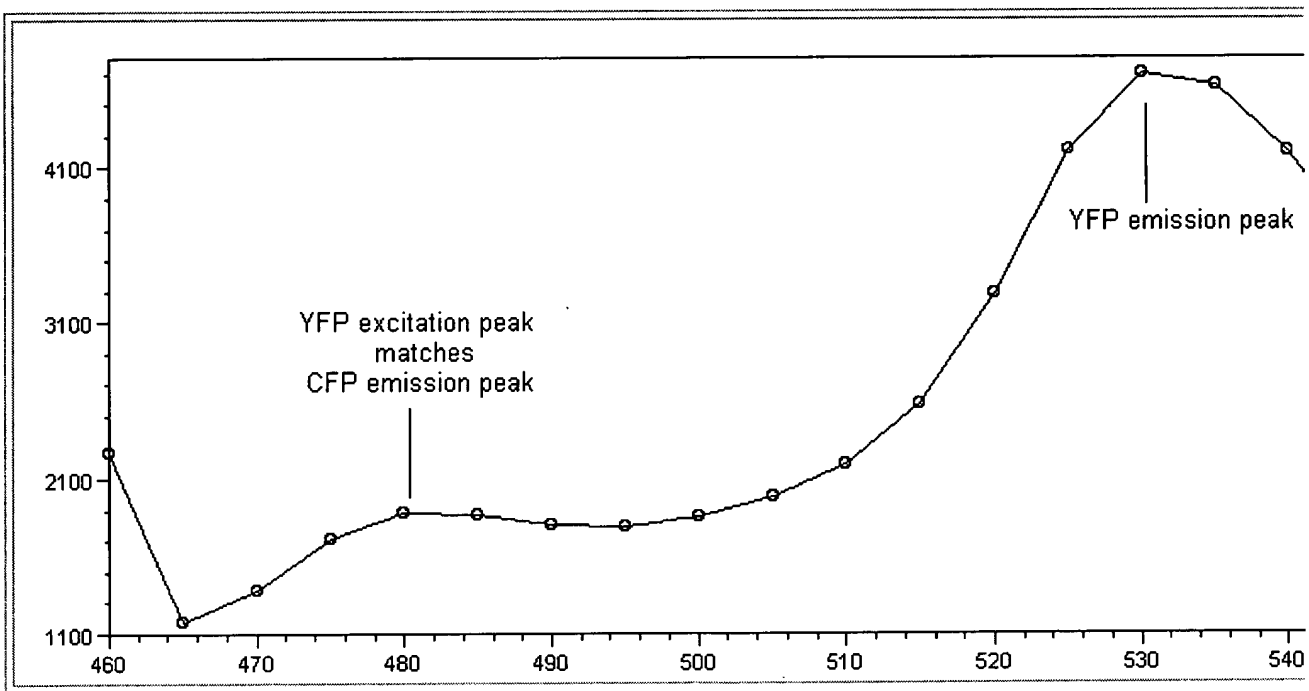
EXHIBIT A

where f_D is the normalized donor emission spectrum, and ε_A is the acceptor extinction coefficient. If either the donor or the acceptor is freely rotating (or both), $\kappa^2 = 2 / 3$ is assumed. On this condition, the R_0 value is determined only by the combination of the donor and acceptor molecules.

Applications

In fluorescence microscopy, fluorescence confocal laser scanning microscopy, as well as in molecular biology, FRET is a useful tool to quantify molecular dynamics in biophysics, such as protein-protein interactions, protein-DNA interactions, and protein conformational changes. For monitoring the complex formation between two molecules, one of them is labeled with a donor and the other with an acceptor, and these fluorophore-labeled molecules are mixed. When they are dissociated, the donor emission is detected upon the donor excitation. On the other hand, when the donor and acceptor are in close proximity (1-10 nm) due to the interaction of the two molecules, the acceptor emission is predominantly observed because of the intermolecular FRET from the donor to the acceptor. For monitoring protein conformational changes, the target protein is labeled with a donor and an acceptor at two loci. When a twist or bend of the protein brings the change in the distance or relative orientation of the donor and acceptor, FRET change is observed. If a molecular interaction or a protein conformational change is dependent on ligand binding, this FRET technique is applicable to fluorescent indicators for the ligand detection.

The most popular FRET pair for biological use is a cyan fluorescent protein (CFP)-yellow fluorescent protein (YFP) pair. Both are color variants of green fluorescent protein (GFP). While labeling with organic fluorescent dyes requires troublesome processes of purification, chemical modification, and intracellular injection of a host protein, GFP variants can be easily attached to a host protein by genetic engineering. By virtue of GFP variants, the use of FRET techniques for biological research is becoming more and more popular.



Example of FRET between CFP and YFP (Wavelength vs. Absorbtion): a fusion protein containing CFP and YFP excited a wavelength. The fluorescent emission peak of CFP overlaps the excitation peak of YFP. Because the two proteins are adjac other, the energy transfer is significant–a large proportion of the energy from CFP is transferred to YFP and creates a much emission peak. (Data: Used with permission from Isaac Li of IBBME, University of Toronto)

FRET is also a common tool in the study of reaction kinetics.

A different, but related, mechanism is the energy transfer of Dexter type.

References:

Joseph R. Lakowicz, "Principles of Fluorescence Spectroscopy", Plenum Publishing Corporation, 2nd edition (July 1, 1999)

Retrieved from "http://en.wikipedia.org/wiki/Fluorescence_resonance_energy_transfer"

Categories: Molecular biology | Cell imaging

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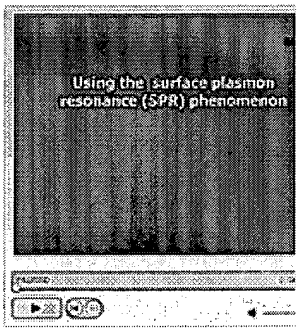
Following an interaction

Biacore systems utilize the natural phenomenon of surface plasmon resonance (SPR) to perform protein interaction analysis. Biacore's technology is non-invasive, label free and delivers high quality results in rea time.

Using the surface plasmon resonance (SPR) phenomenon



Using the surface plasmon resonance (SPR) phenomenon
Requires Flash Player 7 | 28sec

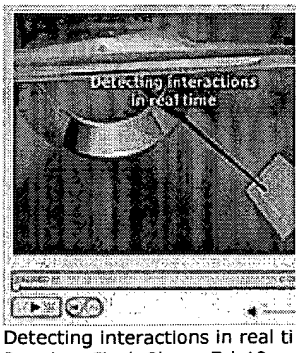
In Biacore systems, the surface plasmon resonance (SPR) phenomenon occurs when polarized light, under conditions of total internal reflection, strikes an electrically conducting gold layer at the interface between media of different refractive index: the glass of a sensor surface (high refractive index) and a buffer (low refractive index).

A wedge of polarized light, covering a range of incident angles, is directed toward the glass face of the sensor surface. Reflected light is detected within a Biacore system.

An electric field intensity, known as an evanescent wave, is generated when the light strikes the glass. This evanescent wave interacts with, and is absorbed by, free electron clouds in the gold layer, generating electron charge density waves called plasmons and causing a reduction in the intensity of the reflected light.

The resonance angle at which this intensity minimum occurs is a function of the refractive index of the solution close to the gold layer on the opposing face of the sensor surface.

Detecting interactions in real time



Detecting interactions in real ti
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As molecules are immobilized on a sensor surface, the refractive index at the interface between the surface and a solution flowing over the surface changes, altering the angle at which reduced-intensity polarized light is reflected from a supporting glass plane.

The change in angle, caused by binding or dissociation of molecules from the sensor surface, is proportional to the mass of bound material and is recorded in a sensorgram.

When sample is passed over the sensor surface, the sensorgram shows an

EXHIBIT B

increasing response as molecules interact. The response remains constant if the interaction reaches equilibrium. When sample is replaced by buffer, the response decreases as the interaction partners dissociate.

Complete profiles of recognition, binding and dissociation are generated in real time. From these profiles, data such as specificity, affinity, kinetic behavior and sample concentration can be determined.
For most applications, a dextran matrix covering the gold layer enables molecules to be immobilized to a sensor surface and provides a hydrophilic environment for interactions. Surface specificity is determined by the nature of the immobilized molecule.

Since light does not penetrate the sample, interactions can be followed in colored, turbid or opaque samples. No labels are required and detection is instantaneous.

Home > Technology > Introduction > Following an interaction

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